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Classical Methods for Studying the Structure of Cells

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ANNOTATION

This review is intended to give a general idea of the basic methods for studying the structure of cells and bacteria. This direction is of great importance for any clinician and is gaining immense popularity around the world due to its accuracy and cost-effectiveness. This review considers such areas of methods as cytomorphology, cytophysiology, cytochemistry and cytogenetics.

Keywords: Cytomorphology; Cytophysiology; Cytochemistry; Cytogenetics

Introduction

Cytomorphology, which studies the features of the structural organization of the cell and its components, still remains the main direction of cell research, since neither the physiological nor the chemical aspect of the analysis of cell vital activity is possible without a solid morphological foundation. The principle of interdependence of structure and function is the basis of any serious cytological study. Neglect of cytomorphology inevitably leads to misconceptions [1,2]. The cytologist's constant companion, the light microscope, still does not lose its importance for studying the structure of the cell. In a number of cases, light microscopy serves as a necessary addition to the most modern instruments, which have a much higher resolution. However, the advances in cytomorphology of the last two decades are associated primarily with the development of electron microscopy. The creation of electron microscopy, which increased the resolution of the microscope by almost 100 times or more, brings modern cytomorphology to the borders to a new direction – to molecular morphology [2]. Electron microscopy has greatly expanded the horizons of our vision. The most important components of the cell, which previously seemed to be structureless formations, now appeared before us in the form of complexly organized structures. Mitochondria, for example, which since the end of the 19th century have been described as structureless rods or granules, were built from a complex system of membranes that form internal partitions. The ideas about the «intracellular mesh apparatus» of the Golgi, which appeared as a complex of membranes, vacuoles and microbubbles, were completely revised. Electron microscopy made it possible not only to clarify the organization of previously known organelles, but also to discover completely new structures. Not a single modern work on cytology, protein secretion and synthesis is now complete without consideration of submicroscopic structures – the endoplasmic reticulum and ribosomes, the existence of which was not even assumed by classical cytology. Electron microscopy finally made it possible to discover that the organization of a number of intracellular components is based on a common structural component, an elementary three-layer membrane [1,3].

The study of this membrane allows us to imagine its macromolecular organization. In a fit of passion for a new method, researchers often forget the sad lessons of the past. We are not guaranteed that the scourge of light microscopy – artifact – will not

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spread to electron microscopy. You can already see the symptoms of this. The «compression artifact» is well known, i.e., the compaction of individual components of the cell as a result of dehydration and pouring into methacrylate. On the myelin sheath of the nerve, it was found that the distance between its layers after fixation decreases to 120 A, and after treatment with lipid solvents (alcohol, acetone) and pouring into methacrylate, it slightly increases (140–150 A), but does not reach natural dimensions (170– 180 A), which were detected by vital microscopy in polarized light. Other artifacts associated with the preparation methods are also known: ruptures and swelling of plant cell walls. One can hope that the artifacts of electron microscopy will be gradually revealed and, just as in light microscopy, we will learn to bypass these reefs [1-3]. Electron microscopy has another disadvantage. The need to examine ultrathin sections in the vacuum of an electron microscope forces the cytologist to study the "corpses" of cells that were previously fixed and poured into sealing media. Attempts to create vital electron microscopy by introducing a gas chamber into the microscope, unfortunately, do not yet go beyond the production experiment. Therefore, vital light microscopy remains an essential addition to electron microscopy in cytomorphological studies. As the resolution (8–10 A) of electron microscopy increases, the cytomorphologist intrudes more and more into the field of molecular morphology, linking cytology with biochemistry. Indirect methods of ultrastructural analysis, such as polarizing microscopy and X-ray diffraction analysis, also lead to this area of molecular organization of the cell. Both of these methods do not make it possible to directly see ultrastructures, but the data obtained in this way make it possible to indirectly judge the intramolecular organization of structures, the orientation of molecules in them, and the distance between them [3,4].

Polarizing microscopy is based on the different refraction of polarized light by different components of cells and tissues. In some of them, light propagates at the same speed regardless of the plane of polarization (isotropic structures), in others (anisotropic structures), the propagation velocity of polarized light depends on its direction along the long or transverse axis of the structure. Many biological objects (for example, myofibrils, ciliated cilia, fission spindle threads), which are characterized by a strict molecular orientation, are anisotropic and have the property of birefringence. In a polarizing microscope, a beam of plane polarized light incident on such structures is decomposed into two beams polarized in mutually perpendicular planes. One of these rays ("ordinary") obeys the usual laws of light refraction, while the other ("extraordinary") passes through anisotropic structures, lagging behind the ordinary beam. Thus, upon exiting the object, both beams are in different phases. This phase shift (the amount of delay of one beam relative to the other) is measured in a polarizing microscope and serves as a measure of birefringence. The value of the delay of one beam relative to the other (G) is determined by the refractive indices (pe, rho) of both beams and the thickness of the object (I) according to the formula: G = (re–ro) • I (in millimicrons or fractions of a wavelength) [5]. If the refractive index along the structure is greater than in the transverse direction, then one speaks of positive birefringence, with reverse ratios – of negative birefringence. Separate structures (myofibrils, fission spindle threads), in which the bonds between molecules are characterized by an asymmetric arrangement, have their own (crystalline) birefringence. Another type of birefringence is structural birefringence, which occurs due to the location of asymmetric submicroscopic particles in a medium with a different refractive index. Cell structures formed by oriented protein molecules (for example, fibers) have their own positive birefringence. Elementary intracellular membranes built from lipid molecules that are perpendicular to protein molecules have their own positive birefringence, which becomes negative after lipid extraction. Nucleoprotein filaments also have negative birefringence. The nature of the polarized light refraction, the value of anisotropy, in combination with the change in these optical parameters after extraction with fat solvents, to a certain extent, make it possible to judge the molecular organization of the structure [5,6].

The method of X-ray diffraction analysis makes it possible to determine not only the orientation of molecules, but also the distance between them. This method is based on X-ray diffraction, which occurs when radiation meets molecules that form a spatial lattice in a substance. In x-ray diffraction analysis, a parallel beam of x-rays is passed through an object, and the diffraction pattern is recorded on a photographic plate located behind the object. A number of concentrically arranged rings, arcs (with a non-oriented molecular structure), spots or dots (with the correct orientation of the particles) are found on the photographic plate. According to the width of the arcs and their location, the particle sizes and the distances between them are calculated [7]. X-ray diffraction analysis is still relatively little used in cytology. However, a number of interesting data have already been obtained by this method. The most significant advances have been made in studying the structure of proteins and nucleic acids. The classical scheme of the structure of deoxyribonucleic acid (DNA), proposed by Watson and Crick, was built on the basis of X-ray diffraction analysis. No matter how deeply we penetrate into the structural organization of the cell, no matter how perfect research methods we create, the main task of cytomorphology remains the study of the living cell. Therefore, vital microscopy is always an essential addition to ultrastructural research methods, even if it does not yet provide such a high resolution. Intravital microscopy allows not only to avoid artifacts, but also to study the dynamics of cell structures [1,7].

The dark-field condenser is widely used to study the colloidal properties of the cytoplasm. By illuminating the cell in the dark-field condenser with side beams (a principle based on the "Tyndall phenomenon"), intracellular structures, even slightly differing in refractive index, become clearly distinguishable due to different luminescence in the general dark field. A very convenient method

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for studying living cells is phase contrast microscopy. The phase-contrast device gives a contrast image of cells and their components in a bright field of view. The essence of the method lies in the fact that the phase shifts of light waves formed when passing through intracellular structures are converted by a phase-contrast lens into light vibrations of different amplitudes, which are perceived by the eye as differences in illumination. Phase contrast microscopy has played an important role in the study of the cell. Suffice it to recall that the long-term discussion about the reality of the Golgi complex was completed thanks to phase contrast and electron microscopy. The most interesting results are obtained by the combination of phase-contrast microscopy with microcinema [7,8]. Luminescent microscopy provides great advantages for studying living cells. Luminescence (or fluorescence) is the glow of structures excited by light energy. Since the radiation of an object is always longer wavelength than the exciting light, ultraviolet rays (300–400 tr) or the blue-violet part of visible light are usually used for luminescence microscopy. Cells and tissues in a fluorescent microscope against a general dark background look differently colored in color and in intensity of luminescence. A number of intracellular formations (for example, inclusions of vitamins A and B2, lipids, porphyrin pigment) have their own (primary) luminescence without additional processing. Other cell components that do not have this property can also give different luminescence after preliminary staining (secondary luminescence) with luminescent dyes – fluorochromes (acridine orange, fluorescein, phloxin, etc.) [9].

Due to its color imaging and high contrast ratio, fluorescence microscopy is a good way to study living cells. This method is also important for cytochemical studies. Secondary luminescence with acridine orange, along with the classical methods of staining a fixed preparation (Felgen's reaction, Unn-Brachet staining), has become one of the main methods for studying the localization of DNA and RNA in a cell. This technique has found wide application in the cytodiagnosis of tumors. Cytophysiology studies the functions of the cell and its components. The cytophysiologist focuses on the processes of cell movement, its protective reactions, nutrition, secretion, excretion, accumulation of various substances, permeability, cell excitation, its reproduction, reactions to external influences. Of particular interest are the functions of intracellular structures and the intracellular regulation of these functions. Much attention is paid to the study of the influence of the whole organism on the activity of individual cells and on the regulation of intracellular processes by the organism. For cytophysiology, the main research method is the experimental method. The most convenient object of cytological experiments is tissue culture outside the body. This method, proposed by Garrison (1907) and later by Karel (1912), consists in placing tissue pieces in a nutrient medium (blood plasma and embryonic extract) on a cover slip, which is mounted over a special glass slide with a hole (the "hanging" method). drops"). Cells grow on the surface of the plasma clot, forming a growth zone along the periphery of the explant. In recent years, synthetic nutrient media for tissue culture have been created and the method of single-layer tissue cultures has been developed. In this case, the cells are cultivated in test tubes, at the bottom of which a piece of coverslip is placed. Cells deposited on the coverslip are used for morphological studies. For tissue culture, either standard transplantable strains (human fibroblasts and amniotic cells; cells obtained from tumors HeLa, Hep-2, etc.) are used, or cells are isolated from the tissue by preliminary trypsinization and the cell suspension is placed in a test tube with a nutrient medium (primary culture). When working with cells outside the body, it must always be taken into account that the artificial conditions of tissue culture do not correspond to the conditions of the whole organism (switching off neurohumoral regulation, possible changes in cell reactivity). However, as a model for studying individual intracellular processes, tissue culture is an indispensable method, although it requires verification of the obtained data on tissue cells of the body [1].

To study the dynamics of the cell and the processes occurring in it (for example, phagocytosis, movement, division, etc.), microfilming is used. Using a time-lapse device, slow-motion (with slow-flowing changes) or accelerated (with fast-flowing processes) shooting is performed and the film is viewed at the usual frequency (24 frames per second). The time-lapse filming method is not only a method of documentation, but, above all, a good method for studying the dynamics of processes in living cells [10]. As in physiology, injections of various substances into a cell, removal or transplantation of its individual structures are widely used in cytophysiological experiments. Micromanipulation (micromanipulation) serves these purposes. Operations on the cell are carried out using a special device – a micromanipulator. With the help of this device, it is possible to remove parts of a cell, introduce various substances with a micropipette, transplant parts of one cell into another, measure electrical potentials (microelectrodes), etc. In modern micromanipulators, the movements of the surgeon's hands are transmitted by pneumatic devices to microinstruments. Microgyrgy (for example, experiments with nuclear transplantation) has provided a lot of important information for solving such problems as the relationship between the nucleus and cytoplasm, the genetic functions of the nucleus, and a number of other issues. In recent years, in microsurgery, the mechanical micromanipulator has begun to be supplanted by another method of operations on the cell, proposed back in 1912 by S. S. Chakhotin. He developed a method of local cell damage using a narrow beam of ultraviolet rays. The use of the beam microprick method made it possible to elucidate, for example, the role of the nucleolus in cytoplasmic protein synthesis, the role of the kinetochore in the movement of chromosomes during mitosis [1].

Cytochemistry studies the localization of chemicals in a cell, the metabolic processes of a cell, and the chemical changes that underlie its functions. Cytochemical studies not only reveal the picture of the chemical topography of the cell, but also reveal a number of features of

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the chemical dynamics of the cell and its individual components. There are four ways to study the chemical characteristics of a cell. Methods of visual cytochemistry consist in reactions between the substance of the cell and certain chemical reagents, and as a result of such a reaction, a microscopically visible reaction product (usually colored) is formed. The intensity and localization of the reaction product is determined visually by microscopic examination of the drug. Currently, we have a large number of different cyto- and histochemical reactions that allow us to detect proteins and amino acids, carbohydrates, lipids and nucleic acids, and some enzymes. Cytochemical reactions are mostly modifications of analytical chemistry reactions. However, unlike the latter, in addition to specificity, a number of additional requirements are imposed on histochemical reactions:

1. The appearance of a clearly visible precipitate or bright and specific staining as a result of the reaction, which can be distinguished on thin sections: under a microscope.
2. The exact location of the reaction product in the place of localization of the substance under study (non-diffusing reaction product); prevention of diffusion 19 of the test substance after taking the material is partially achieved by using freeze-drying for fixation (quick freezing at a temperature of about -160°C followed by vacuum drying) and the frozen state replacement method (fixation of frozen tissue with chilled ethanol); for the same purpose, when cutting, a deep-cooling knife and a cryostat are used.
3. Higher sensitivity of the cytochemical reaction, since the amount of substances that are studied in the cell is usually very small.
4. The chemical reaction should not destroy the structure of the cell; in this regard, many reactions of analytical chemistry are not applicable, requiring the use of concentrated acids, alkalis, or heating to a high temperature [11].

A prerequisite for cytochemical studies is the conduct of control reactions (removal, blocking of a substance or its active groups in the cell, or exclusion of the substrate from the incubation medium when determining enzymes). Severe conditions imposed on cytochemical reactions narrow the ranges of visual cytochemistry [1,11]. In recent years, methods of visual cytochemistry have been combined with electron microscopy. Electronic cytochemistry allows the localization of chemicals on individual cell ultrastructures. The range of reactions of electron cytochemistry is even more limited, since the reaction must necessarily form a product that strongly absorbs electrons. At present, such reactions have been developed for the determination of nucleic acids, succinate dehydrogenase, diaphorases, adenosine triphosphatase, acid and alkaline phosphatases, cholinesterase, and glycogen [11,12]. However, all methods of visual cytochemistry give only a qualitative description of the chemical features of the cell. The current level of development of cytology persistently requires a transition to accurate, objective quantitative indicators of chemical statics and cell dynamics. Assessment of chemical changes in the cell "by eye" is gradually becoming a thing of the past. Quantitative cytochemical studies are carried out by physical methods for studying the chemical composition and their combination with visual cytochemistry [13]. Physical methods of cytochemistry are based on the characteristic absorption spectrum of certain substances in visible, ultraviolet or infrared light and on the dependence of light absorption on the concentration of the substance. reactions. The principle of the method is based on the fact that the intensity of absorption of rays is proportional to the concentration of the substance at the same thickness of the object (the Lambert–Beer law). A device for cytophotometry consists of a light source, a filter, a microscope, and a photometer with a photomultiplier onto which the image of the cell is projected. The intensity of light transmission through the cell or the reciprocal of the optical density (extinction) is determined, and these indicators are compared with standard samples or with other cells [12-14].

For the same purposes, ultraviolet microscopy with cytophotometry is widely used. Ultraviolet microscopy, along with an increase in the resolution of the microscope (0.1 r), opens up wide possibilities for chemical studies of the cell. In the ultraviolet region, bands of strong absorption of ultraviolet rays of a number of substances that are transparent in visible light are concentrated. So, nucleic acids selectively absorb rays with a wavelength of 260 tr, cyclic amino acids (tryptophan, tyrosine) absorb rays with a wavelength of 270–295 hpr, and acyclic amino acids with a wavelength of 220–230 hpr. The presence of selective absorption of ultraviolet rays makes it possible, by microscopy at different wavelengths, to isolate such substances and details that are lost in visible light due to the proximity of their refractive indices to the refractive index of the medium. The combination of ultraviolet microscopy with cytophotometry also allows quantitative analysis [1,11,12]. E. M. Brumberg proposed two methods of ultraviolet microscopy: visual and photographic. In the first of them, a fluorescent screen is placed in the image plane of the preparation, the fluorescence color of which depends on the wavelength. In the second method, the color transformation of the image is achieved in such a way that three pictures are taken from one place of the preparation at different wavelengths of ultraviolet rays, and the positives are examined in a chromoscope, in which the pictures are illuminated with red, green and blue light. The chromoscope reduces three images into one color image [15]. Since a sufficiently strong absorption of ultraviolet rays gives a relatively limited range of biological substances (nucleic acids, some amino acids), the further development of this method consists in combining ultraviolet microscopy with visual cytochemical reactions that change the nature of the absorption of ultraviolet rays of the studied substances. For the purposes of cytochemical analysis, the luminescent microscopy discussed above is also used.

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The use of fluorochromes makes it possible to study the distribution of proteins, lipids, mucopolysaccharides, and nucleic acids in the cell. Luminescent microscopy became especially widespread for the purposes of cytochemistry after the development of the Koons method, which used antibodies labeled with fluorescein isocyanate to detect antigens in cells. The combination of immunobiological reactions with luminescent microscopy allows deeper insight into the protein metabolism of the cell. In this way, it was found that the protein molecules involved in the construction of the fission spindle preexist in the interphase cell. Using the method of fluorescent proteins, the localization of myosin in discs A of the myofibril and the presence of adrenocorticotropic hormone in the basophilic cells of the pituitary gland were shown, and a number of other interesting cytochemical features of different cells were revealed [16].

The amount of various substances in a cell is usually related to its volume or dry weight. The determination of the mass of dry matter of the cell is carried out using interference microscopy, which allows an accuracy of determination up to 0.5 DO-12 g. Interference microscopy is based on the same principle as phase contrast microscopy. In an interference microscope, an additional light wave is superimposed on the image of a cell. This is done in such a way that a light beam that has passed through the object and has therefore undergone some change in phase meets another light beam from the same source that has passed by the object (reference beam) and has the original phase. When these beams meet, they interfere and an interference image of the object appears. The phase shift of one beam relative to another is measured with a rotating analyzer, and from these data the difference between the refractive indices of the object and the medium is determined, and then the dry weight of the object is calculated [12]. One of the most important and accessible methods of cytochemistry is autoradiography, based on the use of radioactive isotopes with α-, β- or γ-radiation, which has the ability to restore the silver bromide of photographic emulsions. After the introduction of the isotope into the cell, the preparation is covered with a photographic emulsion and after a while the emulsion is developed in the usual way. Comparing the illuminated areas of the emulsion (autograph) with the corresponding areas of the cell, it is possible to accurately localize the inclusion sites of the isotope. For biological research, isotopes that give β-radiation (C14, P32, etc.) are usually used. In recent years, compounds labeled with tritium (3H) have become the most widely used in autoradiography. The β-particles that arise from the decay of tritium have a much lower energy than the radiation of labeled carbon or phosphorus, and therefore the resolution of such autographs is the highest. Particularly interesting data were obtained in this way in the study of the nucleic acid metabolism of the cell. The use of a labeled DNA precursor (3H-thymidine) made it possible to trace chromosome replication. The combination of autoradiography with electron microscopy (electron autoradiography) opened the way to the study of the chemical dynamics of cell ultrastructures. The data obtained by this method on the synthesis of enzymes in the process of secretion deserve special attention. Autoradiography generally serves as an indispensable method for studying the localization of biochemical processes occurring in the cell and its individual components [17].

Cells often use conventional biochemical or microchemical methods to study chemical changes. But to carry out such studies, the organs are first destroyed (homogenized), and then their homogenate is subjected to fractionation. The latter is carried out by differential centrifugation. By centrifuging the homogenate for different times at different speeds, first the heavier components are sequentially isolated, and then, upon repeated centrifugation, the lighter components of the cell. Thus, it is possible to isolate different components of the cell: nucleus, nucleolus, mitochondria, Golgi complex and other intracellular structures. The possibility of obtaining a large amount of material and the applicability of proven biochemical research methods have led to the fact that cell fractionation has become one of the main methods for studying the chemical characteristics of a cell. Despite the fact that important biochemical facts underlying many modern cytological concepts have already been obtained in this way, the evaluation of these data requires caution, since the fractionation method is not free from a number of significant drawbacks:

1. Morphological and biochemical artifacts associated with the process of destruction itself and the phenomena of autolysis.
2. Since the whole organ or part of it is usually subjected to homogenization, a heterogeneous group of cells is analyzed simultaneously, and the result is attributed to a certain "average cell".
3. It is not always possible to isolate pure fractions.
4. During fractionation, uncoupling of enzymes and substrates inevitably occurs and, thus, the activity of enzymatic processes changes. The number of such considerations could be increased, but what has already been said is enough to justify a cautious assessment of the results of cell fractionation and the conclusion that this method of research should be controlled by other methods of cytochemistry [18].

Cytogenetics is one of the sections of cytology that develops at the intersection of cytology with genetics. This area of research is devoted to the study of the cytological foundations of heredity and variability. The main object of cytogenetic studies is the chromosome, its morphology, biochemistry and physiology. As the chemical features of the chromosome are studied, cytogenetics gradually merges with molecular genetics, which studies the synthesis and replication of DNA, the molecular mechanisms of its genetic function, and the genetic control of protein synthesis [19-21]. In addition to the main areas of cytology, briefly described above, there are a number of other areas devoted to the development of particular problems of this discipline, for example, radiation cytology, cytoecology [22].

Conclusion

The differentiation of cytology into separate areas, due to the capacity and specificity of the methods of each of these sections, is fraught with one-sidedness and the prospect of accumulating non-generalized facts. Therefore, it seems that the further development of this science lies on the path of the development of "synthetic cytology", that is, a comprehensive study of the cell by representatives of different directions. However, one-sided biochemical studies are also not enough to solve complex cytological issues. So, complex cytology, which comprehensively considers the main manifestations of cell life, should be developed by the friendly efforts of researchers of various profiles: cytomorphologists, cytochemists and physicists.

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